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PLANAR REFERENCE ELECTRODE

FIELD OF THE INVENTION

5 The present invention relates to a planar reference electrode and a process for fabrication thereof and more specifically to the reference electrode including substrate (4); electrode connection part (1); electrodes formed on the plate
10 (3); insulating membrane (2); inner reference solution (5); junction (7 or 9); and protecting membrane (6, 8 or 9), in which the junction comprises porous material such as cotton thread, glass fiber, cellulose nitrate, cellulose acetate, filter paper;
15 polymer-based porous membrane; or capillary type.

BACKGROUND OF THE INVENTION

20 Electrochemical methods such as potentiometry or voltammetry have been commonly applied in the analytical laboratories to determine specific chemical components contained in clinical, environmental or industrial samples. However, the samples collected from remote sampling sites often require special
25 pretreatment to prevent them from contamination or degradation during transportation to the laboratories. The laboratory-based instruments is expensive to

operate, and should be managed by the technicians specially trained in the area. In addition to the aforementioned problems, some clinical samples, e.g., blood for in vitro tests, would be collected in 5 smaller volume in order to reduce the patient's uncomfortable feelings and shock. For these reasons, on-spot-monitoring or point-of-care-testing (POCT) with convenient and economic hand-held devices is becoming popular to expedite the analytical tasks, 10 while reducing the laboratory operational costs and errors by non-specialists.

To design portable electroanalytical devices, miniaturization of the electrochemical sensor system 15 including reference and working electrodes should be attained. Numerous schemes have been proposed to accomplish miniaturized working electrodes, and many of them are now commercially available, while reference electrodes have not been extensively 20 developed for miniaturization thereof.

An ideal miniaturized reference electrode requires several characteristics, such as insensitive response to the sample's composition change, providing 25 a constant potential which is characteristic of a reversible redox couple reaction within the half cell. The reversibility of a fast redox reaction ensures the

recovery of the same potential even after the passage of electric currents through the reference cell. The reference cell including insoluble metal salt (e.g., Ag/AgCl) provides constant potential by exchanging a 5 cation or anion which is normally contained in a high concentration of reference electrolyte, either in aqueous solution or in a hydrogel medium, which is in contact with the sample solution through a junction. Accordingly, when such a reference system is 10 miniaturized, it is necessary to implement a micro junction, easily activated reference electrolyte, and stable insoluble metal salt on a chip. To use the miniaturized reference electrode for an electrochemical POCT system, fast stabilization of the 15 potential and reproducibility are prerequisite.

Several types of miniaturized reference electrodes have been proposed previously. A reference electrode comprising a screen printed silver/silver 20 chloride electrode, a layer of low temperature melting glass paste or a silicone polymer paste containing potassium chloride, and a hydrophobic polymer membrane that can form micro junctions by hydration has been reported (Cranny, A. W. J.; Atkinson, J. K., Meas. Sci. 25 Technol. 1998, 9, 1557-1565). Unfortunately, the presoaking time for this reference system takes more than an hour and the potential is unstable, although

it provides long operational lifetime. Another example of small reference electrode was prepared by the following steps: placing an inner reference electrolyte in the form of hydrogel on a silver/silver 5 chloride electrode; and covering the hydrogel layer with a hydrophobic polymer membrane with a small opening (Lauks, I. R., US patents no. 4,993,048). This reference electrode system provides a constant potential only for a short period of time (a few 10 minute) as the small volume of inner reference electrolyte depletes into the sample solution through the junction, and changes the inner salt concentration. However, the formation of a small exposure, which is typically a few micrometers, in the process of 15 mounting an outer membrane is not easy and often results in malfunction of the reference electrode system due to the sealed pore. In addition, there is a possibility that large particulates contained in sample may block the junction. Such an abnormality can 20 not be predicted in advance and lowers the reliability of the measurement. Miniaturized reference electrodes based on field effect transistor (FET) were also proposed (Potter, W.; Dumschat, C.; Cammann, K., *Anal. Chem.*, 1995, 67, 4586-4588): the top of the ion- 25 selective layer is coated with another layer containing solid potassium perchlorate. Since potassium perchlorate is not readily soluble in

aqueous solutions, the perchlorate activity in the aqueous surface layer is equal to that of a saturated solution and leads to a constant potential. However, to deposit the potassium perchlorate layer, this 5 system requires a well (diameter 2 mm, and height 1.5 mm) affixed on the chip and is not suitable for mass fabrication.

In order to develop planar reference electrodes 10 that are suitable for mass fabrication with stable reference potential for an elongated period of time, porous material such as cotton thread, glass fiber, cellulose nitrate, cellulose acetate, filter paper and any material that can induce capillary action; porous 15 polymer membrane; or viscous inner reference solution enclosed in a well formed with a punched film layer has been provided as the junction of the reference electrode. The present invention discloses that the aforementioned reference electrodes maintain stable 20 potentials and provide a relatively short activation period. Hence, it can be employed both in potentiometry and voltammetry. The reference electrodes proposed in this invention can be easily miniaturized in planar structure and appropriate for 25 mass fabrication.

SUMMARY OF THE INVENTION

The present invention describes a miniaturized planar-type reference electrodes that can be used both in potentiometry and voltammetry and a method for 5 fabricating it. Further objectives and advantages of the present invention will appear hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

Features and advantages of the present invention 10 may be understood more clearly from the following detailed description in conjunction with the accompanying drawings, in which;

15 **Fig. 1a** shows the front view of the planar reference electrode of the present invention in which the junction comprises porous substance or a capillary formed on the substrate.

Fig. 1b shows the inner structure of Fig. 1a.

20 **Fig. 1c** shows the cross sectional view of Fig. 1a through A - A' line.

Fig. 1d shows the cross sectional view of Fig. 1a through B - B' line.

Fig. 1e shows the cross sectional view of Fig. 1a through C - C' line.

25

Fig. 2a shows the front view of the planar reference electrode of the present invention, in which

the junction comprises porous polymer membrane.

Fig. 2b shows the internal structure of Fig. 2a.

Fig. 2c shows the cross sectional view of Fig. 2a through A - A' line.

5 **Fig. 2d** shows the cross sectional view of Fig. 2a through B - B' line.

Fig. 2e shows the cross sectional view of Fig. 2a through C - C' line.

10 **Fig. 3** shows the graph of potentiometric response curve of Ag/AgCl electrode to Cl^- .

a : Ag/AgCl electrode screen printed on a polyester substrate

15 b : Ag/AgCl electrode screen printed on polycarbonate substrate

Fig. 4 shows the pH response of the planar reference electrode of the present invention employing,

20 a : the planar reference electrode of example 1 (junction : cotton thread ; inner reference solution : 98.5% glycerol solution of saturated NaCl),

b : the planar reference electrode of example 4 (junction : capillary type ; inner reference solution : 85% glycerol with 3 M KCl (volume ratio; 2 : 1)),

25 c : the planar reference electrode of example 6 (junction : capillary type; inner reference solution : polymeric glue saturated with KCl),

d : the planar reference electrode of example 8
(junction : cellulose nitrate membrane ; inner
reference solution : PVP (polyvinyl pyrrolidone)
polymer hydrogel prepared by using 2 M KCl solution
dissolved in 0.2 M CaCl_2).

Fig. 5 shows the response of the planar reference electrode to the change in Cl^- ion concentration employing,

10 a : the planar reference electrode of example 1,
b : the planar reference electrode of example 4,
c : the planar reference electrode of example 6,
d : the planar reference electrode of example 8.

15 **Fig. 6** shows the activation period of the planar
reference electrode of the present invention employing,
a : the planar reference electrode of example 1,
b : the planar reference electrode of example 4,
c : the planar reference electrode of example 6,
d : the planar reference electrode of example 8.
20

Fig. 7 shows the potential stability of the planar reference electrode of the present invention with time employing,

25 a : the planar reference electrode of example 1,
b : the planar reference electrode of example 4,
c : the planar reference electrode of example 6,

d : the planar reference electrode of example 8.

Fig. 8 shows the Cl^- ion response of the Ag/AgCl working electrode with respect to the conventional reference electrode and the planar reference electrode of the present invention employing,

a : the conventional reference electrode (Orion sleeve-type double junction),

b : the planar reference electrode of example 1,

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10      c : the planar reference electrode of example 2
(junction : glass fiber ; inner reference solution :
98.5% glycerol solution of saturated NaCl),
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d : the planar reference electrode of example 3
(junction : cellulose nitrate; inner reference
15 solution : 98.5% glycerol solution of saturated NaCl),

e : the planar reference electrode of example 4,

f : the planar reference electrode of example 6,

g : the planar reference electrode of example 8.

20 **Fig. 9** shows the hydrogen ion response of hydrogen ion-selective solid-state type working electrode with respect to the conventional reference electrode and the planar reference electrode of the present invention employing,

a : the conventional type reference electrode,

b : the planar reference electrode of example 4,

c : the planar reference electrode of example 6,

d : the planar reference electrode of example 8.

Fig. 10 shows the sodium ion response of Na^+ ion-selective solid-state type working electrode with
5 respect to the conventional type reference electrode
and the planar reference electrode of the present
invention employing,

10 a : the conventional type reference electrode,
b : the planar reference electrode of example 4,
c : the planar reference electrode of example 6,
d : the planar reference electrode of example 8.

15 **Fig. 11** shows the calcium ion response of Ca^{2+} ion-selective solid-state type working electrode with
respect to the conventional type reference electrode
and the planar reference electrode of the present
invention employing,

20 a : the conventional type reference electrode,
b : the planar reference electrode of example 5,
(junction : capillary type ; inner reference solution :
85% glycerol solution of saturated AgNO_3),
c : the planar reference electrode of example
7 (junction : capillary type; inner reference solution :
polymer glue of saturated AgNO_3),
25 d : the planar reference electrode of example 8.

Fig. 12 shows the urea and ammonium ion response

of urea and ammonium ion-selective solid-state type working electrode with respect to the planar reference electrode of the present invention,

5 a : urea response with the planar reference electrode of example 4,

b : ammonium response with the planar reference electrode of example 4,

c : urea response with the planar reference electrode of example 8,

10 d : ammonium response with the planar reference electrode of example 8.

15 **Fig. 13** shows the voltage-current response curve of the screen-printed carbon paste electrode to ferri/ferrocyanide redox reaction with respect to the planar reference electrode of the present invention and the saturated calomel electrode employing,

a : the planar reference electrode of example 4,

b : conventional saturated calomel electrode.

20

● **Drawing Reference Numerals**

1 : electrode connection part

2 : insulating membrane

3 : electrode

25 4 : substrate

5 : inner reference solution

6, 8 : protection membrane

7 : junction

9 : porous polymer membrane (functions as both junction and protection membrane)

5

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

In accordance with the present invention, the object of the present invention could be accomplished by providing reference electrode including plate (4); 10 electrode connection part (1); electrode (3); insulating membrane (2); inner reference solution (5); junction (7 or 9); and protecting membrane (6, 8 or 9), wherein the junction comprises porous material such as cotton thread, glass fiber, cellulose nitrate, 15 cellulose acetate, filter paper and any material that can produce capillary action; porous polymer membrane; or a film with micro capillary.

Fig 1a, 1b, 1c, 1d, 1e, 2a, 2b, 2c, 2d and 2e show the examples of the planar reference electrode of 20 the present invention. **Fig. 1a, 1b, 1c, 1d and 1e** show the examples of the planar reference electrode in which junction is composed of porous substance or a film with a line of micro capillary. **Fig. 2a, 2b, 2c, 2d and 2e** show the examples of the planar reference electrode in 25 which junction is composed of porous polymer membrane. As depicted in **Fig. 2a, 2b, 2c, 2d and 2e**, an extra protection membrane is not needed since the porous

polymer membrane itself also plays a role of a protection membrane when porous polymer membrane is used as a junction.

5 The planar reference electrode of the present invention is characterized in that the junction is composed of porous substance such as cotton thread, glass fiber, cellulose nitrate, cellulose acetate, filter paper and any other material that can provide
10 capillary action; porous polymer membrane; or a film with a line of micro capillary.

15 The junction plays a role in transferring ions through the micro channels between sample solution to be tested and the inner reference solution, thereby the two solutions is electrically connected. On the other hand, the junction must not corrupt the sample solution or inner reference solution by moving inner reference solution toward the outside sample solution and vice versa.

20 The junction of the present invention comprises porous material, porous polymer membrane or capillary. If a micro capillary formed on a thin film is used as the junction, the performance of the reference electrode is not dependent on the type of junction-
25 forming material employed.

 The planar reference electrode of the present invention improves the stability of electrode potential

and reduces the activation period of the electrode by using the aforementioned junction-forming methods and materials. Advantageously, the reference electrode having porous polymer membrane as a junction has
5 shorter hydration period than other reference electrodes that employ hydrophobic outer membrane. One of the preferred porous polymer material is cellulose nitrate. In addition, the reference electrode with micro capillary provide faster activation time since
10 the junction needs not to be hydrated. Such a electrode can be prepared conveniently due to simplicity of the junction-forming method.

The inner reference solution (5) comprises
15 hydrogel containing electrolytes which is composed of 85-99% weight% of glycerol solution; 1-10 weight% of agar solution; polymer glue; or other water soluble polymers.

The inner reference solution of the present
20 invention exploits a viscous solution or a soluble polymer containing a high concentration of electrolytes. Since the inner reference solution is made of the viscous solution, the inner reference solution need not be hydrated and provide rapid activation while
25 minimizing the contamination of sample solution due in part to the retarded diffusion of inner reference electrolytes. The activation time of the reference

electrode can be reduced further by adding hygroscopic substance such as CaCl_2 with a proper concentration when hydrogel is used as an inner reference solution.

As a soluble polymer forming hydrogel, poly(vinyl pyrrolidone) (PVP) is preferable. The hydrogel can be prepared by dissolving soluble polymer with organic solvent and drying.

As an electrolyte, silver nitrate or perchloric acid is preferred, if silver is used as an electrode material, KCl or NaCl is preferred, if Ag/AgCl is used, NaOH or KOH is preferred, if mercury/mercury oxide is used.

The plate (4) is formed of alumina, glass plate or thermostable plastic substance, preferably polyester or polycarbonate. Polycarbonate is favored as the plate material in the present invention if the cellulose nitrate is used as the outer membrane component.

The protection membrane (6, 8 or 9) is formed of plastic substance including polyester or porous polymer membrane as cellulose nitrate.

Porous polymer such as cellulose nitrate has micelle structure, uniform porosity and hydrophilic property. Advantageously, it can be exploited not only as protection membrane and but also as polymer

membrane type junction in the reference electrode of the present invention.

The electrode (3) can consist of metal layer such 5 as Ag, Pd, Cu, Pt and any conducting material. Also, the electrode can consist of metal layer/insoluble metal layer such as Ag/AgCl, and Hg/HgO. By using the screen printing technique, the above electrode material can be easily manufactured onto the plate.

10

Insulating membrane (2) is formed onto the electrode material (3) layer excluding electrode site and electrode connection part (1).

15

Meanwhile, preferably the distance between the junction site and the working electrode of the planar reference electrode is relatively long when used in potentiometry, and shorter when used in voltammetry to reduce the IR drop problem.

20

Furthermore, the present invention also provides processes for preparing the planar reference electrode.

Particularly, in the case that the junction of the planar reference electrode is composed of porous 25 material or capillary type, the process for fabricating the planar reference electrode comprises 7 stages as follows;

(1) forming electrode connection part (1) on plate (4);

(2) forming electrode (3) on plate (4) by using the screen printing method;

5 (3) forming insulating membrane (2) layer by screen printing onto the conductors (3) formed at step 2, excluding electrode site, capillary channel and connection sites;

10 (4) forming insoluble metal salt layer on the electrode site;

(5) placing a thin film that can provide a well around the electrode site and a capillary onto the substrate;

15 (6) placing inner reference solution (5) within the well;

(7) forming protection membrane layer (8) that cover the inner reference solution; and

20 In the case that the junction of the planar reference electrode is composed of porous polymer membrane, the process for preparing comprises 6 stages as follows;

25 (1) forming electrode connection part (1) on plate (4);

(2) forming electrodes (3) on plate (4) by using the screen printing method;

(3) forming insulating membrane (2) layer by

screen printing onto the conductors (3) formed at step 2, excluding electrode site and connection sites (1);

5 (4) forming insoluble metal salt layer onto the electrode;

(5) forming hydrogel layer (5) using soluble polymer containing highly concentrated electrolyte; and

10 (6) forming porous polymer protection membrane (9) on the hydrogel layer using porous polymer covering hydrogel layer completely.

At stage (6), porous polymer protection membrane (9) is made to cover the entire hydrogel layer and then blocked direct contact with sample.

15 Meanwhile, in the case that electrode material consists of metal layer/insoluble metal layer, the metal layer is formed by using the screen printing method and then insoluble metal salt layer can be built by using separate processes. For example, in the case that electrode material (3) is metal Ag and insoluble metal salt AgCl, insoluble metal layer can be formed by using the chemical reaction with $FeCl_3$ or $KCrO_3Cl$ solution. Also, the insoluble metal salt can 20 be overlaid within solution containing Cl^- ion such as HCl , in which constant current (for example 0.4 mA/cm) 25 is used to electroplate the layer for several minutes.

The planar reference electrode of the present invention is insensitive toward the changes in Cl^- , Na^+ , and H^+ concentration in solution, and exhibits a 5 short activation period, typically less than a few minutes. The electric potential is maintained stable for more than 4000 seconds; when the K^+ , Na^+ , NH_4^+ , H^+ , and Cl^- selective electrodes are referenced to the planar reference electrode of present invention, they 10 all exhibit the same potentiometric responses as they are referenced to the conventional reference electrodes.

20 Cyclic voltammetry for the ferri/ferrocyanide redox reaction with respect to the planar reference electrode result in a similar curve obtained with the calomel electrode. Thus the planar reference electrode of the present invention can be used not only in the potentiometry but also in the voltammetry.

25

EXAMPLES

Practical and presently preferred embodiments of 25 the present invention are illustrated as shown in the following Examples.

However, it will be appreciated that those

skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

5 Example 1: Preparation 1 of the planar reference electrode

10 The reference electrode of the present invention was prepared according to the method described below by using porous cotton thread as junction material.

15 First, electric conducting line was built onto the polyester plate (4) by using the conventional screen printing method with silver paste from the region of electrode site (3) and electrode connection part (1). Then insulating membrane (2) layer onto the metal conductor (3) excluding the electrode site and electrode connection part (1) was formed by using the typical screen printing method employing an insulating paste.

20 The above electrode (3) was placed in 0.0175 M concentration of FeCl_3 solution for 20-30 minutes to form insoluble metal salt layer, AgCl , onto the electrode site (3).

25 The polyester was cut out in a size that can cover the electrode site and then punched to form a well (6 mm diameter) that can hold inner reference solution. From one end of the well to the outer

boundary, a straight channel was made to place a junction material.

Porous cotton thread was utilized as junction material and placed on the straight channel of the 5 polyester film. The polyester layer (8) prepared above was glued to the plate (4).

Inner reference solution (5) was made by the following process; preparing 98.5 weight% of glycerol 10 solution and saturated with KCl. And 3 μ l of the inner reference solution was put into the well formed with the polyester layer.

Finally, another polyester film (3) that serves as the outer protection membrane was glued on the top of the well containing polyester (8), which completes 15 the construction of the reference electrode as shown in **Fig. 1.**

Example 2: Preparation 2 of the planar reference electrode

20

The reference electrode was prepared according to the method as described in example 1 by using porous glass fiber (Whatman international Ltd., Maidstone, England) as junction material instead of cotton thread.

25

Example 3: Preparation 3 of the planar reference electrode

The reference electrode was prepared according to the method as described in example 1 by using porous cellulose nitrate (Whatman international Ltd., 5 Maidstone, England) as junction material instead of cotton thread.

Example 4: Preparation 4 of the planar reference electrode

10

The reference electrode with capillary type junction was prepared as described below.

15

The electrode comprising metal layer and insoluble metal layer, electrode connection and insulating membrane was made by using the process as described in example 1.

20

The polyester was cut out in a size that can cover the electrode site and then punched to form a well (6 mm diameter) that can hold inner reference solution. From one end of the well to outer boundary, a straight channel was made by simply cutting a line with sharp blade to form a capillary.

25

Inner reference solution (5) was made by the following process; preparing 85 weight% of glycerol solution and 3 M of KCl solution and mixing with 2 : 1 volume ratio. And 3 μ l of the inner reference solution was put into the well formed by the polyester layer.

Finally, the another polyester film (3) that serves as the outer protection membrane was glued on the top of the well containing polyester (8), which completes the construction of the reference electrode as shown in **Fig. 5. 1.**

Example 5: Preparation 5 of the planar reference electrode

10 The reference electrode was prepared according to the method as described in example 4 by using capillary type junction. But, Ag was utilized as electrode material instead of forming Ag/AgCl layer and 85 wt % of glycerol solution saturated with AgNO₃ as an inner 15 reference solution.

Example 6: Preparation 6 of the planar reference electrode

20 The reference electrode was prepared according to the method as described in example 4 by using capillary type junction. But polymeric glue saturated with KCl was utilized as an inner reference solution.

Example 7: Preparation 7 of the planar reference electrode

The reference electrode was prepared according to the method described in example 4 by using capillary type junction. But Ag was utilized as the electrode instead of Ag/AgCl and a polymeric glue saturated with 5 AgNO₃ as an inner reference solution.

Example 8: Preparation 8 of the planar reference electrode

10 The reference electrode with porous polymer membrane as the outer protection membrane, which also serves as a junction, was prepared as described below.

15 The electrode comprising metal layer and insoluble metal layer, electrode connection and insulating membrane was built onto a polycarbonate plate by using the process described in example 1.

20 Soluble polymer PVP (poly(vinyl pyrrolidone)) was dissolved in 2 M KCl solution (2 wt %) and then 0.2 M CaCl₂ was added to this solution. The above solution was mounted onto the reference electrode site by using an air press-type dispenser and dried at 60 °C for 30 minutes, forming hydrogel layer containing electrolytes of high concentration on Ag/AgCl electrode.

25 90 mg of porous polymer, cellulose nitrate (NC), was dissolved in 1000 µl of organic solvent THF (tetrahydrofuran). NC solution was dispensed onto the hydrogel layer by using an air-press type dispenser and

dried at 60°C for 30 minutes.

By the process described above, hydrogel layer containing electrolytes with high concentration onto Ag/AgCl electrode and porous polymer protection membrane, cellulose nitrate membrane, were made and the planar reference electrode was completed (as shown in 5 **Fig. 2**).

Table 1 shows the composition of components and junction type of the planar reference electrode as 10 prepared in the example 1 - example 8.

<Table 1>

Composition of the planar reference electrode

15

example	electrode	junction type	junction material	inner reference solution	plate /protection membrane
1	Ag/AgCl	porous material	cotton thread	98.5 weight% of glycerol solution saturated with KCl	polyester
2	Ag/AgCl	porous material	glass fiber	98.5 weight% of glycerol solution saturated with KCl	polyester
3	Ag/AgCl	porous material	cellulose nitrate	98.5 weight% of glycerol solution saturated with KCl	polyester
4	Ag/AgCl	capillary	no	98.5 weight% of glycerol solution saturated with KCl	polyester
5	Ag	capillary	no	98.5 weight%	polyester

				of glycerol solution saturated with AgNO_3	
6	Ag/AgCl	capillary	no	liquid pool saturated with KCl	polyester
7	Ag	capillary	no	liquid pool saturated with AgNO_3	polyester
8	Ag/AgCl	porous polymer membrane	cellulose nitrate membrane	hydrogel of 2 weight% PVP, 0.2 M CaCl_2 , 2M KCl	polycarbonate/ cellulose nitrate membrane

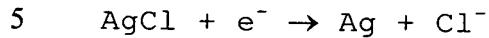
Experimental Example 1: Electrochemical performance of the planar reference electrode with Ag/AgCl electrode - sensitivity toward Cl^- ion

5

We have performed the experiment described below in order to examine the performance of the planar reference electrode prepared in example 1-4, 6 and 8. As shown in **Figure 3.**, The bare Ag/AgCl electrode formed 10 on the substrate exhibited proper sensitivity to 10^{-6} - 10^{-1} M Cl^- ion. To examine the performance of the Ag/AgCl electrodes, their potential responses with respect to the conventional type reference electrode, Orion^R sleeve-type double junction reference electrode 15 (Ag/AgCl electrode, 90-20 (USA, ORION corp.) was measured.

The Ag/AgCl electrodes which was prepared as described in example 1-4 and 6 result in the 53.03 mV/dec of slope to Cl^- ion. And the electrode which was 20 prepared as described in example 8 exhibited 54.6

mV/dec of slope to Cl^- ion. The responses of Ag/AgCl electrode may be explained with the following equations:



$$E = E_{\text{Ag/AgCl}} - (2.303RT/F) \log a_{\text{Cl}^-}$$

E : electric potential

$E_{\text{Ag/AgCl}}$: standard potential of Ag/AgCl

10 R : gas constant

T : absolute temperature

F : Faraday constant

a_{Cl^-} : activity of Cl^-

15 In both cases, the response slopes, 53.03 mV/dec and 54.60 mV/dec, to Cl^- are close to that of the theoretical one, 59.16 mV/dec at the given room temperature.

20 Experimental Example 2: Electrochemical performance of the planar reference electrode - sensitivity toward pH

25 To examine the performance of the planar reference electrodes of the present invention, their potential responses with respect to the conventional type reference electrode, Orion^R sleeve-type double junction reference electrode (Ag/AgCl electrode, 90-20

(USA, ORION corp.) was measured in varying pH condition.

The results are shown in **Fig. 4.**

As shown in **Fig. 4**, all planar reference electrodes of the present invention exhibited 5 insensitive responses to varying hydrogen ion concentration in the pH 3 - 12 range.

Experimental Example 3: Electrochemical performance of the planar reference electrode - sensitivity toward

10 **NaCl**

To examine the performance of the planar reference electrodes of the present invention, their potential responses with respect to the conventional 15 type reference electrode, Orion^R sleeve-type double junction reference electrode (Ag/AgCl electrode, 90-20 (USA, ORION corp.) was measured in varying NaCl concentration. The results are shown in **Fig. 5.**

As shown in **Fig. 5**, all planar reference 20 electrodes of the present invention exhibited insensitive responses to varying Cl⁻ ion concentration in the 10⁻¹ - 10⁻⁶ range.

Experimental Example 4: Electrochemical performance of

25 **the planar reference electrode - activation period**

We have performed the experiment to examine the

activation period of the planar reference electrodes as described below.

The same electrode system with that of the above experimental example 2 was used to measure the 5 activation period of the reference electrodes by immersing them in 0.002 M of Tris-H₂SO₄ buffer solution of pH 7.4.

As shown in **Fig. 6**, the planar reference electrode using porous material as junction were 10 stabilized less than 10 seconds of activation (**Fig. 6a**). The electrodes described in example 1-3, those employing porous junction material, showed similar results. In addition, the capillary-type planar reference electrodes (example 4, 6; **Fig. 6b**, **Fig. 6c**) 15 and the planar reference electrode with porous polymer membrane (example 8; **Fig. 6d**) were also activated less than several seconds.

As described above, the planar reference electrodes of the present invention are suitable to use 20 in clinical and industrial fields, as they require very short activation time within several seconds.

Experimental Example 5: Electrochemical performance of
the planar reference electrode - stability

25

In order to see that the planar reference electrode maintains stable electrochemical property for

an extended period of time, we have examined their stability as described below.

5 The same electrode systems described in example 2 was used and the planar reference electrodes, and they were immersed in 0.002 M Tris-H₂SO₄ buffer solution (pH 7.4) to measure the electric potential variation for more than 4000 seconds.

10 As shown in **Fig. 7**, the planar reference electrode using porous material as junction maintained stable electric potentials for more than 4000 seconds (Fig. 7a). The electrodes described in example 1-3, those employing porous junction material, showed similar results. In addition, the capillary-type planar reference electrodes (example 4, 6; Fig. 7b, Fig. 7c) 15 and the planar reference electrode with porous polymer membrane (example 8) were also maintained stable for more than 4000 seconds.

20 **Experimental Example 6: Comparative experiment for the performance of conventional reference electrode and the planar reference electrodes of the present invention - sensitivity toward Cl ion**

25 In order to compare the potentiometric performance of the planar reference electrode of the present invention with that of the conventional reference electrode, we have performed the experiment

as described below.

The potentiometry electrode system was composed of working electrode (typical Ag/AgCl electrode), reference electrodes (the planar reference electrode of the present invention and conventional planar reference electrode) and volt meter which can measure the potential differences between the working electrode and conventional reference, and between the working and the planar reference electrode. Standard solutions (10^{-6} - 10^{-1} M of NaCl solutions) were used to examine the response characteristics toward Cl^- ion. The results were depicted in **Fig. 8** and listed in Table 2.

<Table 2>

15 Sensitivity toward Cl ion

reference electrode	sensitivity toward Cl ion of working electrode (mV/dec)
Conventional reference	53.02
Example 1	54.88
Example 2	54.13
Example 3	53.39
Example 4	59.20
Example 5	54.61
Example 6	52.79

As shown in **Fig. 8** and table 2, Ag/AgCl working electrode exhibited 53.02 mV/dec of slope to Cl^- ion 20 (**Fig. 8a**) with respect to the conventional reference

electrode. With respect to the planar reference electrodes of the present invention, i.e., the reference electrodes prepared in example 1, 2, 3, 4, 6 and 8, the working electrode exhibited 54.88 mV/dec of 5 slope toward Cl^- ion (example 1, **Fig. 8b**), 54.13 mV/dec (example 2, **Fig. 8c**), 53.39 mV/dec (example 3, **Fig. 8d**), 59.20 mV/dec (example 4, **Fig. 8e**), 54.61 mV/dec (example 6, **Fig. 8f**) and 52.79 mV/dec (example 8, **Fig. 8g**). These results are essentially the same as that 10 observed with the conventional reference electrode system.

Experimental Example 7: Comparative experiment for the performance of conventional reference electrode and the planar reference electrode of the present invention - sensitivity toward H^+ ion (pH)

As described in experimental example 6, similar experiments were carried out to examine the 20 potentiometric performance of the planar reference electrodes of the present invention. Hydrogen ion-selective electrode was prepared with the membrane doped with tridodecylamine (TDDA) on the planar-format electrode. The comparative potentiometric responses of 25 the H^+ -selective electrode with respect to the conventional electrode and planar reference electrodes were shown in **Fig 9**.

As depicted in **Fig. 9**, the slope toward H^+ ion was 53.59 mV/dec with respect to the conventional reference electrode (**Fig. 9a**). With respect to the reference electrode of the present invention, the 5 reference electrode prepared in example 4 showed 54.23 mV/dec of slope (**Fig. 9b**), the reference electrode prepared in example 9, 58.10 mV/dec (**Fig. 9c**), the reference electrode prepared in example 8, 57.58 mV/dec (**Fig. 9d**). They exhibited essentially the same response 10 characteristics compared to that of the conventional reference electrode.

Experimental Example 8: Comparative experiment for the performance of conventional reference electrode and the planar reference electrode of the present invention - sensitivity toward Na^+ ion

As described in experimental example 6, similar experiments were carried out to examine the 20 potentiometric performance of the planar reference electrodes of the present invention. Hydrogen ion-selective electrode was prepared with the membrane doped with 4-tertbutylcalix(4)arene on the planar-format electrode. The comparative potentiometric 25 responses of the H^+ -selective electrode with respect to the conventional electrode and planar reference electrodes were shown in **Fig 10**.

As shown in **Fig. 10**, the response slope toward Na^+ ion was 57.18 mV/dec with respect to the conventional reference electrode (**Fig. 10a**). With respect to the planar reference electrodes of the present invention, the reference electrode prepared in example 4 showed 59.72 mV/dec of slope (**Fig. 10b**), the reference electrode prepared in example 6, 57.28 mV/dec (**Fig. 10c**), the reference electrode prepared in example 8, 56.21 mV/dec (**Fig. 10d**). They exhibited essentially the same response characteristics compared to that of the conventional reference electrode.

Experimental Example 9: Comparative experiment for the performance of conventional reference electrode and the planar reference electrode of the present invention - sensitivity toward K^+ ion

As described in experimental example 6, similar experiments were carried out to examine the potentiometric performance of the planar reference electrodes of the present invention. Hydrogen ion-selective electrode was prepared with the membrane doped with valinomycin on the planar-format electrode. The comparative potentiometric responses of the H^+ -selective electrode with respect to the conventional electrode and planar reference electrodes were shown in **Fig 11.**

As shown in **Fig. 11**, the response slope toward K^+ ion was 57.04 mV/dec with respect to the conventional reference electrode (**Fig. 11a**). With respect to the reference electrode of the present invention, the 5 reference electrode prepared in example 5 showed 55.10 mV/dec of slope (**Fig. 11b**), the reference electrode prepared in example 7, 55.74 mV/dec (**Fig. 11c**), the reference electrode prepared in example 8, 56.70 mV/dec (**Fig. 11d**). They exhibited essentially the same 10 response characteristics compared to that of the conventional reference electrode.

Experimental Example 10: Sensitivity toward urea and ammonium ion

15 In order to examine the sensitivity of the working electrode toward urea and ammonium with respect to the planar reference electrode of the present invention, we have performed the experiment as 20 described below.

Ammonium ion-selective electrode was fabricated with nonactin-doped polymer membrane on the planar electrode. Urea responsive electrode was prepared by immobilizing urease on the top of the ammonium- 25 selective membrane. Experimental setup was similar to that described in experimental example 6. The results are shown in **Fig. 12**.

As shown in **Fig. 12**, the response slope toward urea was 56.2 mV/dec with respect to the reference electrode of example 4 (**Fig. 12a**). The slope toward ammonium was 55.6 mV/dec (**Fig. 12b**). The slope toward urea was 56.2 mV/dec with respect to the planar reference electrode of example 8 (**Fig. 12c**) and the slope toward ammonium was 56.2 mV/dec (**Fig. 12d**). Thus the planar reference electrode of the present invention was is a proper reference electrode system in the measurement of ammonium and urea.

Experimental Example 11 : Cyclic voltammetry (CV) curve
for the feeri/ferrocyanide redox reaction

In order to examine the usefulness of the planar reference electrode of the present invention in voltammetry, we examined the CV for the ferri/ferrocyanide redox reaction employing the planar reference electrode of the present invention.

The voltammetry electrode system was composed of working electrode, supporting electrode, reference electrode and current or potentiostat, which can measure the electric charge. The conventional type reference electrode, saturated calomel electrode was used as a reference electrode to compare with the planar reference electrode of the present invention prepared in example 4, and the carbon paste electrode

as a working electrode and Pt electrode as a supporting electrode. The electrode of the above 3 electrode system was immersed in 1 mM of $K_3Fe(CN)_6$ solution to obtain CV for the oxidation-reduction pair of $K_3Fe(CN)_6^{3-}$ / $K_3Fe(CN)_6^{2-}$. The results were shown in **Fig. 13.**

As shown in **Fig. 13**, the potential difference between the oxidation and reduction peaks was 65 mV (**Fig. 13a**) when the planar reference electrode of the present invention was used. When the conventional saturated calomel reference electrode was used, the potential difference between the oxidation and reduction peaks was also 65 mV (**Fig. 13b**), which was the same value as that obtained with the planar reference electrode of the present invention. In addition, maximum oxidation and maximum reduction currents were also identical in the above 2 cases using different reference electrodes, respectively. However, in the CV of the ferri/ferrocyanide system with the planar reference electrode, the electric potential of the maximum oxidation peak and the maximum reduction peak were shifted toward positive direction of about 20 mV compared to those observed with the conventional saturated calomel reference electrode. This difference is related to the difference in standard potential of the respective reference electrodes, but not in their functions.

As stated above, the planar reference electrode of the present invention was identified to have the same performance with that of the calomel reference electrode and thus can be exploited as a reference 5 electrode system in voltammetry.

INDUSTRIAL APPLICABILITY

The planar reference electrode of the present 10 invention has the junction which is composed of porous material such as cotton thread, glass fiber, cellulose nitrate, cellulose acetate, filter paper and any other material that can induce capillary action; porous polymer membrane; or a capillary embedded in the 15 assembly. The planar reference electrodes of the present invention provide stable electric potential and short activation period, and the reproducibility of the results was outstanding.

In addition, the planar reference electrodes of 20 the present invention have simpler structure than the existing reference electrodes, and can be prepared conveniently. Furthermore, it can be produced cheaply in mass production, and used in both potentiometry and voltammetry. Electrode material and inner reference 25 solution of the planar reference electrode may be changed to the demand of analytical purpose. Therefore, the scope of the planar reference electrode of the

present invention is not limited only to the examples described in this document.

Those skilled in the art will appreciate that the 5 conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such 10 equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.